Abstract

Low back pain affects nearly 80% the population at least once in their lifetime. Intervertebral disc (IVD) degeneration is thought to play a role in the development and onset of low back pain. The center part of the disc, the nucleus pulposus (NP), which supports high compressive loads daily, shows early signs of degeneration, long before the outer part of the disc, the annulus fibrosus (AF) degenerates.

This work is divided into two parts: the first part focuses on the biomechanics of IVD cells in response to cyclic loading, whereas the second part focuses on the selection of a cell carrier for the further *in vivo* delivery of fetal spine cells to induce disc regeneration.

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1. Effects of mechanical stress on IVD cells

To have a better understanding of the biomechanics of IVD, an alginate disk was designed for IVD cell culture (especially NP cells) and mechanical stimulation. IVD cells (NP and AF) were obtained from rat tail harvesting. A comparison study between NP and AF cells was carried out. First, a mechanical and biological characterization of IVD cells seeded in alginate disk was investigated through stiffness of the alginate, as well as cell morphology, cell viability, DNA content. A loading device was used to applied mechanical stimulation on alginate disks. Five different sample groups were analyzed: samples loaded with a cyclic strain (10% or 25%, at 0.5 Hz), with a static strain (10% or 25%) and control unloaded samples. The mechanical stiffness of the alginate disk was then calculated and cellular response to loading was characterized by quantifying cell viability and gene expression of collagen type II (A and A+B), collagen type I, aggrecan, Sox-9 and MMP-2. The effect of disc tissue digestion and alginate embedding were analyzed as well for the different genes.

Characterization of alginate disk gave encouraging results with appropriate cell morphology, DNA content. In comparison with AF cells, NP cells expressed significant higher levels of collagen type II a+b and Sox9 and significant lower levels of collagen of type I, which is consistent with the different properties between AF and NP parts. Moreover, gene expression of NP cells seeded in alginate disk displayed a similar phenotype than in native disc tissue. Mechanical parameters indicated that alginate disks storage modulus was always about 50 fold higher than loss modulus in the range of 26-34kPa for AF and control

groups and with a greater variation for NP cells (13-47kPa). With time, a stress relaxation curve was observed reaching a constant value 450 cycles (900s). Although the stiffness of alginate disk did not seem reproducible, these observations were consistent with the viscoelastic properties of alginate disks. The cell response to load showed that cells remain viable inside the alginate disk for each groups. Gene expression studies demonstrated that NP cells tended to increase the production of every genes after loading, whereas static loading induced the production of collagen type I, collagen type II (A+B), Sox 9 and MMP-2. AF cells, however, tended to increase gene expression of aggrecan, collagen type II and MMP-2 with higher expression obtained for cyclic loading, whereas the expression of collagen type I and Sox 9 decreased in almost all conditions. For NP cells, different levels of aggrecan, collagen type I and MMP-2 expression were observed for the different strain, whereas for AF cells, this tendency was not observed for all genes. In agreement with previous studies, NP cells appeared to be more magnitude dependent than AF cells.

This *in vitro* model demonstrated the possibility to use alginate disk for IVD cells characterization. Enhanced studies with other cell sources, more homogenous cell population, reproducible alginate disk, higher cell density and longer mechanical stimulations will be necessary to refine this model.

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2. Selection of a cell carrier for the in vivo delivery of fetal spine cells

Up to now, there is no effective clinical treatment for disc degeneration. An important field of research is focused on cell therapy to replace the damaged nucleus and keep the annulus intact. In this context, we hypothesize that fetal cells, coming from human fetal spine tissue, could express main nucleus components and be a potential source for intervertebral disc regeneration. Fetal cells provide many advantages for the scale-up of in vitro tests into clinical trials since they are already differentiated and have high expansion potential and low immunogenicity properties. For this work, one sample of fetal spine cells isolated from a 14-week fetal spine tissue was tested.

To characterize fetal spine cells *in vitro*, the cell were cultured in alginate beads. Fetal spine cells showed an increase of type II collagen and aggrecan expressions between day 7 and 14 of culture into alginate beads, whereas type I collagen expression did not appear to increase during culture. Histological observations showed rounded chondrocyte-like cells morphology with GAG-containing matrix around the cell membrane. These first results emphasized the potential of fetal spine cells to express chondrocyte phenotype.

Therefore, an appropriate cell carrier for the *in vivo* injection of the fetal spine cells is needed. Regarding intervertebral disc regeneration, the cell carrier has to possess adequate biological properties for cell survival, cell proliferation and cell differentiation into chondrocyte-like cells. Moreover, it must allow homogenous distribution of cells and nutrient diffusion, as well as production of extracellular matrix components. For future clinical applications, it has to be biocompatible and biodegradable to allow nucleus regeneration, as well as be injectable, reproducible and easy handled.

Based on previous studies, gelatin, chitosan and collagen based biomaterials were chosen as potential cell carriers for the *in vivo* injection of fetal spine cells. Gelatin and gum arabic hydrogel provided non-homogenous results with cell migration outside the gel. In addition, this hydrogel was highly viscous and did not seem to be injectable and reproducible. Chitosan and β-glycerophosphate hydrogel did not form a stable hydrogel at physiological temperature and was thus difficult to use. Atelocollagen hydrogel gave encouraging results for cell survival, cell histology and GAG-containing matrix production. However, for further clinical applications, more extended studies have to be done *in vitro* with enhanced parameters to enlarge these results over a large scale of samples.

These two different studies emphasized two different approaches of cell carrier design, the first for a biomechanical application, the second for a clinical application. Even though applications were different, biological aspects were targeted towards cellular response to new extracellular environment and external factors.